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Myocardial adenosine A_{2a} receptor imaging of rabbit by PET with [^{11}C]KF17837

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Adenosine A_{2a} receptors are found in the endothelia, vascular smooth muscle cells and cardiac myocytes. The properties of a carbon-11 labeled A_{2a} antagonist [^{11}C]KF17837 ([7-methyl-^{11}C](*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine) for myocardial imaging were evaluated by dynamic PET scanning of the myocardium in rabbits. Myocardial uptake of [^{11}C]KF17837 was clearly visualized by PET. The tracer was taken up at a high level by the myocardium immediately after the injection, and the myocardial level of radioactivity gradually decreased. On the other hand, an inactive [^{11}C]Z-isomer of [^{11}C]KF17837 showed a very low myocardial uptake and the myocardium was not visualized with a selective A_1 antagonist [^{11}C]KF15372. By co-injection with carrier KF17837 or a xanthine type A_{2a} antagonist 7-chlorostyrylcaffeine (CSC), the myocardial uptake of [^{11}C]KF17837 was completely blocked. The effect of non-xanthine A_{2a} antagonists ZM 241385 and SCH 58261, which have a higher affinity than CSC, was smaller than that of the CSC. The effect of weak antagonists caffeine and alloxazine or a xanthine type A_1 antagonist KF15372 on the radioactivity level was small. It is concluded that PET with [^{11}C]KF17837 can image myocardial adenosine A_{2a} receptors.

Key words: [^{11}C]KF17837, xanthine, adenosine A_{2a} receptors, rabbit myocardium, positron emission tomography

INTRODUCTION

ADENOSINE is an endogenous modulator of synaptic functions in the central nervous system (CNS) as well as in the periphery. The effect is mediated by two major subtypes of receptors; adenosine A_1 receptors which exhibit higher affinity for adenosine and inhibit adenylyl cyclase, and A_2 receptors which exhibit lower affinity for adenosine and stimulate adenylyl cyclase. Recent advances in molecular biology and pharmacology have demonstrated the presence of at least five subtypes i.e., A_1, A_{2a}, A_{2b}, A_3 and A_4 receptors. They act via GTP binding proteins and are coupled not only to adenylyl cyclase but also to ion channels and phospholipases. The current status of the adenosine receptors has been reviewed.[1-7]

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In the cardiovascular system, the adenosine A_1 receptors are present on cardiac myocytes. Activation of the A_1 receptors has been reported to elicit bradycardia, depression of myocardial contractility and reduction of impulse conduction velocity. The A_2 receptors are present on the endothelium and on the vascular smooth muscle cells, mediating the endothelium-dependent and -independent vasodilation, respectively. Although conflicting data exist regarding the presence and function of A_2 receptors on the cardiac myocytes, recently Xu et al. have clearly shown that A_{2a} receptors are expressed and are functionally coupled to the stimulation of cAMP accumulation and cardiac contractility in adult rat ventricular myocytes.[8]

During the last decade, many neuroreceptors in humans and other animals have been visualized *in vivo* by positron emission tomography (PET) with appropriate radioligands. The PET technique may offer an opportunity to understand the regulation and properties of the adenosine receptors in the cardiovascular system. Recently Suzuki and co-workers have developed a number of xanthine

type adenosine antagonists selective for A_1 or A_{2a} receptors.[9–13] We have labeled some of them with carbon-11 as potential PET ligands for the two adenosine receptor subtypes of the CNS: [^{11}C]KF15372 ([3-propyl-^{11}C]8-dicyclopropylmethyl-1,3-dipropylxanthine)[14,15] and its methyl and ethyl derivatives[16] for adenosine A_1 receptors, and [^{11}C]KF17837 ([7-methyl-^{11}C](*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine) for the adenosine A_{2a} receptors.[17] In rodent studies, these compounds were found to be promising PET ligands in the CNS. On the other hand, only [^{11}C]KF17837 was taken up by the heart at a higher level than other organs, and the murine heart was visualized by whole-body *in vivo* imaging with a gamma camera.[17] In the present study we report the successful imaging of the myocardium of the rabbit by PET with [^{11}C]KF17837, and characterize the properties of the compound as a PET ligand for mapping myocardial adenosine A_{2a} receptors.

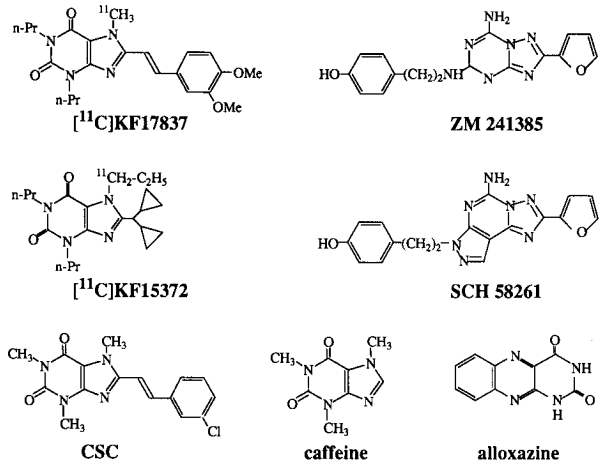

Fig. 1 Chemical structure of [^{11}C]KF17837 and adenosine antagonists used in the present study.

Table 1 Affinity of adenosine antagonists for the adenosine receptors

	Affinity Ki (nM)		Selectivity	Ref.
	A_1	A_{2a}	A_{2a}/A_1	
KF17837	62#	1.0*	62	(12,13)
Z-isomer	>10000#	860*	> 12	(13)
KF17837S¶	390#	7.9*	49	(12,13)
CSC	28000##	54*	520	(20,21)
ZM 241385	510##	0.91**	560	(27)
SCH 58261	121#	2.3*,†	53	(29)
KF15372	3.0##	430**	0.0070	(9)
caffeine	29100#	48100**	0.60	(24)
alloxazine	5250#	2720**	1.9	(24)

Radioligands used as A_1 ligands were N^6-[^{3}H]cyclohexyladenosine# and N^6-[^{3}H](S-2-phenylisopropyl)adenosine##. Radioligands used as A_2 ligands were [^{3}H]2-[p-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H]CGS 21680)* and [^{3}H]5′-*N*-ethylcarboxamidoadenosine ([^{3}H]NECA)**. ¶Equilibrated state of an active *E*-form and an inactive *Z*-form of KF17837. †Kd = 0.70 nM.[29]

Table 2 Injected tracers and adenosine antagonists in the successive PET scanning of rabbits

Experiment	PET scanning			
	1st	2nd	3rd	4th
1. Tracer	[^{11}C]KF17837 31 MBq/0.44 nmol	[^{11}C]KF17837 44 MBq/19.0 nmol		
Co-injected antagonist*		KF17837		
2. Tracer	[^{11}C]KF17837 39 MBq/0.85 nmol	[^{11}C]KF15372 5.4 MBq/0.69 nmol	[^{11}C]KF17837 49 MBq/0.61 nmol	
Co-injected antagonist*			KF17837	
3. Tracer	[^{11}C]KF17837 40 MBq/0.56 nmol	[^{11}C]Z-isomer 35 MBq/0.55 nmol	[^{11}C]KF17837 21 MBq/7.8 nmol	
Co-injected antagonist*			KF15372	
4. Tracer	[^{11}C]KF17837 47 MBq/2.7 nmol	[^{11}C]KF17837 48 MBq/6.0 nmol		
Co-injected antagonist*		CSC		
5. Tracer	[^{11}C]KF17837 15 MBq/0.26 nmol	[^{11}C]KF17837 41 MBq/6.5 nmol	[^{11}C]KF17837 29 MBq/47 nmol	[^{11}C]KF17837 43 MBq/1.3 nmol
Co-injected antagonist*				caffeine
6. Tracer	[^{11}C]KF17837 39 MBq/0.86 nmol	[^{11}C]KF17837 35 MBq/4.0 nmol	[^{11}C]KF17837 13 MBq/2.8 nmol	
Co-injected antagonist*		alloxazine	ZM 241385	
7. Tracer	[^{11}C]KF17837 36 MBq/1.9 nmol	[^{11}C]KF17837 36 MBq/0.49 nmol		
Co-injected antagonist*		SCH 58261		

*All doses of co-injected antagonists were 2000 nmol.
The same rabbits were used for experiments 1 and 3, and experiments 4 and 5.

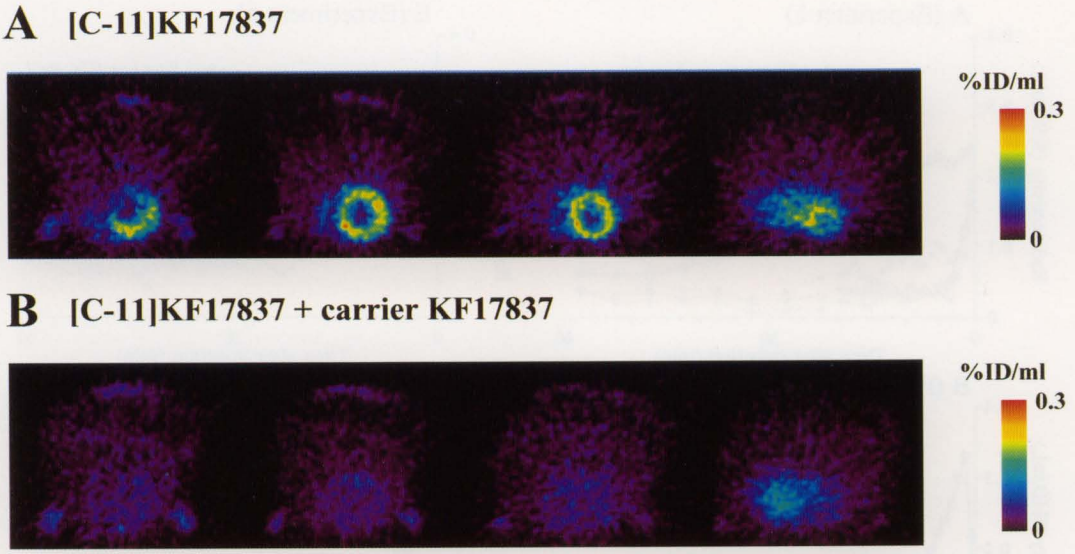

Fig. 2 Images of the chest region of a rabbit by PET scanning with adenosine A_{2a} antagonist [^{11}C]KF17837 (A) and [^{11}C]KF17837 with carrier KF17837 (B). The images were acquired for 30 min starting at the injection.

MATERIALS AND METHODS

[^{11}C]KF17837 was prepared by the reaction of desmethyl KF17837 and [^{11}C]methyl iodide as described.[17] In some experiments, the [^{11}C]KF17837 was further isomerized to [^{11}C]*Z*-isomer (74% of inactive *Z*-form and 26% of active *E*-form) under visible light, which was analyzed by HPLC immediately before injection.[17] An adenosine A_1 ligand [^{11}C]KF15372 was prepared as described.[14] KF17837, desmethyl KF17837, KF15372 and despropyl KF15372, as well as other A_{2a} antagonists including CSC (7-chlorostyrylcaffeine), ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl)phenol) and SCH 58261 (5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyridine) were prepared by Kyowa Hakko Kogyo Co. Caffeine was purchased from Sigma (St. Louis, MO) and alloxazine (benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) was obtained from Aldrich Chemical Company, Inc. Chemical structures of ^{11}C-labeled tracers and antagonists are shown in Figure 1. The affinities of adenosine antagonists for adenosine A_{2a} and A_1 receptors are summarized in Table 1.

PET Study

The experimental protocols are summarized in Table 2. Five male rabbits (1.9–2.1 kg) were used for seven experiments in the present study. Experiments 1 and 3 and experiments 4 and 5 were conducted on the same individual within a week.

The rabbits were anesthetized with isoflurane (1.5–2.5% in air), and were placed in the prone position on a holder made of polyacrylate.[18] [^{11}C]KF17837 was intravenously injected through the ear vein, and PET scanning was performed over a period of 60 min (base line). After the radioactivity decayed out, a second tracer was injected together with or without one of the adenosine antagonists, and a 60 min PET scanning was again performed. Two to four PET examinations were carried out successively on the same rabbits at 90 to 120 min intervals with co-injection of various adenosine antagonists. The PET camera was a model SHR 2000 (Hamamatsu Photonics, Hamamatsu, Japan). The camera consists of four-ring detectors and acquires seven slices with a resolution of 4.0 mm FWHM in the transaxial plane.[19] The scanning schedule was either 60 1 min frames or 20 1 min frames and then eight 5 min frames. A ring-shaped region of interest was placed over the myocardium, and the myocardial time-activity curve was obtained in the same region both for the base line and the loading experiments.[18] Corresponding blood time-activity curves were obtained by placing a region of interest over the left ventricular chamber. The decay-corrected radioactivity value was expressed as a percentage of the injected dose per m*l* tissue volume (%ID/mL).

The animal studies were approved by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Gerontology.

RESULTS

Figure 2A shows typical images of the chest region of rabbits obtained by PET scanning with [^{11}C]KF17837. A ring-shaped image of the myocardium was clearly visualized. The uptake by the liver was also visualized, but the lungs were scarcely observed. On co-injection of carrier KF17837 (Experiments 1 and 2), the myocardial image disappeared (Fig. 2B). The time-activity curves in

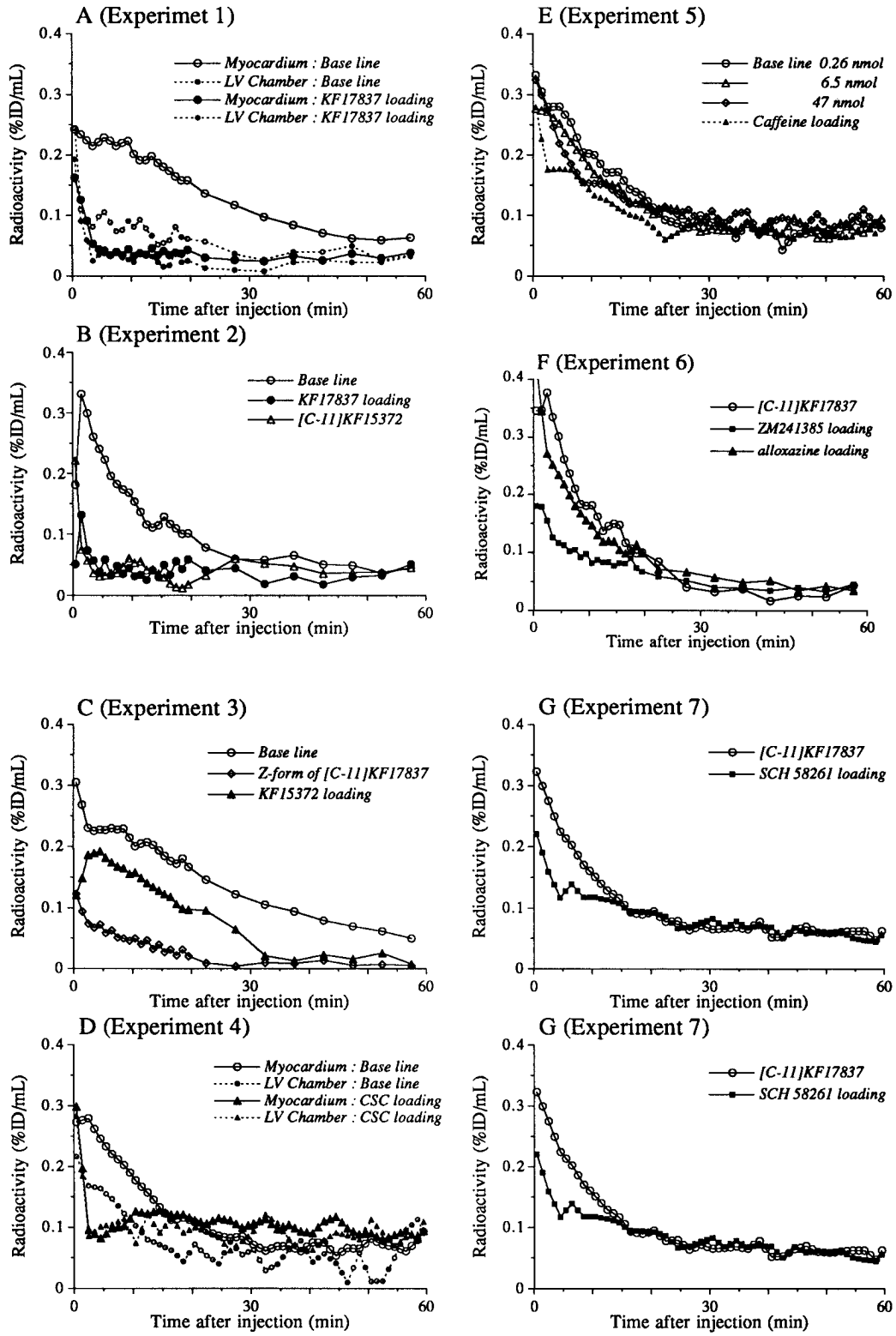

Fig. 3 Time-activity curves on the rabbit myocardium after intravenous injection of [^{11}C]KF17837 with or without co-injection of adenosine receptor antagonists, or other tracers ([^{11}C]KF15372 for Experiment 2, scan 2 and [^{11}C]Z-isomer for Experiment 3, scan 3). Experimental protocols are summarized in Table 2. The radioactivity level is expressed as the percent of injected dose per mL tissue volume of the myocardium obtained from a ring form region of interest. The time-activity curves over the left ventricular chamber were shown in Experiments 1 and 4, and were not added in other experiments to prevent the complicated drawing.

experiment 1 are shown in Fig. 3A. In the first scan (base line), immediately after the initial peak due to blood radioactivity spillover, a high myocardial uptake of the tracer was observed for the first 10 min (corresponding to approximately 0.22 of %ID/mL), and then the myocardial level of radioactivity was gradually decreased. After 30 min the myocardial image had almost disappeared. The radioactivity level over the left ventricular chamber rapidly decreased for the first 5 min and then gradually decreased. On the other hand, when carrier KF17837 was co-injected, the myocardial level of radioactivity was rapidly decreased to a background level at 5 min. The time-activity curves over the myocardium and left ventricular chamber were equivalent.

In the second experiment (Fig. 3B), reproducibility of the effects of carrier KF17837-loading was observed. In contrast with [^{11}C]KF17837, no retention of adenosine A_1 antagonist [^{11}C]KF15372 was observed in the heart. The time-radioactivity curve of [^{11}C]KF15372 was similar to that of carrier-loading [^{11}C]KF17837.

In the third experiment (Fig. 3C), after injection of the *Z*-isomer of [^{11}C]KF17837 which contained only 26% of active *E*-form, the initial uptake of radioactivity was lower than that of the base line, and then the myocardial level of radioactivity was gradually decreased. When adenosine A_1 antagonist KF15372 was co-injected, the radioactivity level was slightly lower, and gradually decreased in a similar way to the base line.

On co-injection of xanthine type A_{2a} antagonist CSC which has 50 times lower affinity for the A_{2a} receptors than KF17837 (Table 1), the radioactivity level was rapidly decreased (Experiment 4, Fig. 3D) similar to that of carrier-loaded [^{11}C]KF17837.

The carrier amount of [^{11}C]KF17837 in the range from 0.26 to 47 nmol did not affect the myocardial level of radioactivity (Experiment 5, Fig. 3E). The effect of a weaker non-selective antagonist caffeine on the radioactivity level of base line was small.

Co-injection of a weak non-xanthine type antagonist alloxazine scarcely decreased the radioactivity level of the base line (Experiment 6, Fig. 3F). Two strong non-xanthine type A_{2a} antagonists, ZM 241385 and SCH 58261, slightly lowered the radioactivity level (Experiments 6 and 7, Figs. 3F and 3G), but the effect was smaller than xanthine type antagonists KF17837 and CSC (Figs. 3A, 3B and 3D).

Among the five rabbits, the base line time-activity curve was slightly different (Fig. 3H). On the other hand, excellent base line reproducibility was found within the same individuals in Experiments 1 and 3 (Figs. 3A and 3C) and Experiments 4 and 5 (Figs. 3D and 3E).

DISCUSSION

The present study has clearly demonstrated that carbon-11 labeled selective adenosine A_{2a} antagonist [^{11}C]KF17837 is a potential PET ligand for mapping adenosine A_{2a} receptors in the myocardium. Several findings in the successive PET measurements in the same rabbits support this conclusion.

[^{11}C]KF17837 was rapidly taken up by the myocardium at a high level, whereas a selective adenosine A_1 ligand [^{11}C]KF15372[14] showed no retention on the myocardium. The uptake of the inactive *Z*-isomer of [^{11}C]KF17837, which actually contained 74% of inactive *Z*-form and 26% of active *E*-form was very low (Fig. 3B).

The myocardial uptake of [^{11}C]KF17837 was completely blocked by co-injection with an excess amount of KF17837 (Figs. 3A and 3B). In a preliminary study, the myocardial uptake of [^{11}C]KF17837 in mice was reduced dose-dependently, and two-thirds of the uptake was blocked at the dose of 1.4 μmol/kg body weight at 15 min after injection of the tracer (data will be presented elsewhere). The uptake was also blocked with xanthine-type adenosine A_{2a} antagonist CSC (Fig. 3D). CSC is currently used as a selective adenosine A_{2a} antagonist for pharmacological studies, but its affinity for the A_{2a} receptors is weaker than KF17837.[20,21] Although an adenosine A_1 antagonist KF15372 slightly decreased the myocardial level of radioactivity from the base line, this reduction may be explained by the presence of its affinity for the A_{2a} receptors (Ki, 430 nM, Table 1).

As indicated above, the present study demonstrated that a selective adenosine A_1 ligand [^{11}C]KF15372 showed no retention on the myocardium in spite of its potential for mapping adenosine A_1 receptors in the CNS.[14] Because it is known that the adenosine A_1 receptors are present on the cardiac myocytes, the reason for this phenomena is not clear. A possible explanation is that the specific activity of [^{11}C]KF15372 was not so high in visualizing the A_1 receptors with low density on the myocytes compared with the CNS.[22]

It is known that selectivity of KF17837 is 28 times higher for the A_{2a} receptors than for the A_{2b} receptors.[12] Because a selective adenosine A_{2b} antagonist is not available, we used alloxazine as an A_{2b} antagonist (A_{2a}/A_{2b}, 0.41),[23] but this compound did not affect the radioactivity level of the base line, which may be explained by its low affinity (Ki, 1100 nM) for A_{2a} receptors.[24] Therefore, we did not clearly assess in the present study whether [^{11}C]KF17837 binds to the A_{2b} receptors of the heart.

As shown in Fig. 3H, the myocardial base line time-activity curves of five rabbits were slightly different from each other. A possible explanation is that the time-activity curves reflect the individual difference in A_{2a} receptor densities in the myocardium because high reproducibility was found in two rabbits (Experiments 1 and 3, and Experiments 4 and 5). Although the carrier doses in the range from 0.26 to 47 nmol did not greatly changed the time-activity curves (Experiment 5, Fig. 3E), it may be that the receptor binding was affected by the administrated doses because of the relatively low density of

receptors in the peripheral organs compared with the CNS. Burns et al. reported that the binding sites of an A_2 antagonist [^{3}H]5′-*N*-ethylcarboxamidoadenosine ([^{3}H]NECA) in the heart were 36 times lower than the striatum in an *in vitro* binding assay.[24] Recently Peterfreund et al. reported less expression of adenosine A_{2a} receptor mRNA in the human heart than in human caudate.[25]

Blood clearance of the tracer was very rapid when assessed by the time-activity curves over the left ventricular chamber in which the radioactivity is overestimated by the spillover of the myocardial radioactivity. In a preliminary study we found the labeled metabolites of [^{11}C]KF17837 in the plasma and brain tissue of mice. The kinetics of plasma radioactivity and the labeled metabolites in the plasma and heart should be measured to quantitatively assess the myocardial adenosine A_{2a} receptors by PET with [^{11}C]KF17837. Furthermore, because the tracer was rapidly taken up by the heart, it would also be elucidated whether the myocardial uptake of the tracer is flow-limited or not. Anyhow, diagnosis of ischemia and other myocardial diseases by PET with [^{11}C]KF17837 is of great interest because of the cardiovascular function of adenosine receptors.

The present *in vivo* study represents a noticeable profile for the pharmacology of adenosine A_{2a} receptors. A number of xanthine-type adenosine antagonists have been developed as caffeine analogs,[26] and ZM 241385[27,28] and SCH 58261[29,30] have been recently proposed as non-xanthine-type antagonists with high affinity for the A_{2a} receptors. In *in vitro* membrane binding assays, the affinity of ZM 241385 and SCH 58261 is 60 times and 20 times, respectively, higher than that of xanthine-type CSC (Table 1) but the present study showed that the blocking effect of ZM 241385 and SCH 58261 on the myocardial uptake of [^{11}C]KF17837 was smaller than that of CSC. Although the radioactivity level rapidly reached the background level within 5 min after the injection due to the blockade with CSC and KF17837, it gradually decreased to the background level due to the blockade with ZM 241385 and SCH 58261. A likely explanation for the discrepancy between the *in vitro* affinity and the effectiveness for the reduction of myocardial uptake of [^{11}C]KF17837, is that xanthine type and non-xanthine type antagonists may recognize different binding sites besides the common binding site(s) within the same A_{2a} receptor *in vivo*. A detailed study on the blocking effects of various xanthine-type and non-xanthine-type compounds on the myocardial uptake in mice will be reported elsewhere. Another possiblity is that the pharmacological effects of these adenosine A_{2a} antagonists on the myocardial blood flow may produce different time-activity curves.

It is reported that adenosine A_{2a} receptors are present on the endothelium and the myocytes. Because of a lack of available A_{2a} selective radioligands and because of the relatively lower receptor densities in the peripheral organs than in the CNS, so far the regulation and properties of the myocardial adenosine A_{2a} receptors have not been well understood. The radiolabeled A_{2a} ligand KF17837 will therefore be a useful probe not only for PET studies but also for pharmacological studies.

In conclusion the present study suggests that PET with [^{11}C]KF17837 can image adenosine A_{2a} receptors of the heart. The A_{2a} receptor-selective radioligand can also offer the opportunity to further elucidate characterization of the adenosine receptors present on the endothelial and cardiac myocytes.

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